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Investigation of angiogenic factors in obese rats exposed to low oxygen pressure

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Abstract

Objective: Obesity, which is one of the most important health problems of today's people, remains current due to the risks of illness it brings due to the increase rate in the world.

Material and Methods: Male Sprague Dawley rats were used in our study of obesity. Rats were divided into four groups as standard diet/ normal oxygen, standard diet/low oxygen, high-fat diet/normal oxygen and high-fat diet / low oxygen. For the study, a special cage with a low oxygen level of 17-18% was made in a closed system. After achieving the desired 25% weight increase in obese group rats, blood, liver, lung, white adipose tissue and brown adipose tissue were obtained from the rats. In these tissues, adrenomedullin, hypoxic inducible factor 1-α (HIF1-α) and matrix metalloproteinase-II (MMP-II) levels were measured by ELISA.

Results: According to our results, there was a significant increase in adrenomedullin, HIF1-α and MMP-II in white adipose tissue, and adrenomedullin and MMP-II in brown adipose tissue. It was found that the amount of HIF1-α increased significantly in liver and lung tissues.

Conclusion: According to the metabolic status of adipose tissue, it is thought that the effect of adrenomedullin, HIF1-α and MMP-II can increase vascularization of brown adipose tissue and provide energy consumption.

Keywords: Hypoxia, Obesity, HIF1-α, MMP-II, Adrenomedullin

Introduction

Hypoxia indicates that the arterial oxygen concentration is lower than normal. Hypoxia is a life-threatening risk factor for organisms, and reducing oxygen prevents biochemical reactions (1). The World Health Organization (WHO) has defined obesity as "fat accumulation that adversely affects the health of other organs" (2). Obesity, which has become one of the most serious health problems of people today, continues to be updated due to increased disease risks. In all countries of the world, obesity has negative health effects, social and economic effects on both individuals and living communities (3). Obesity is the cause of many diseases and decreases the quality of life and the life span. Obesity appears as an important increasing health problem affecting more than 20% of the western population. It significantly increases the risks of obesity, type-2 diabetes, hypertension, coronary heart disease, stroke, liver failure, dementia, obstructive sleep apnea, and various cancers. Therefore, diseases associated with obesity and / or diseases such as atherosclerosis, diabetes and cancer significantly affect quality of life (4). Obesity is an energy metabolism disorder that is characterized by physical and mental problems due to the accumulation of too much fat in the body.

Obesity is a multi-factor phenomenon caused by inequality between calorie intake and use (5). The amount of fat in the body is usually indicated by the body mass index (BMI) and is calculated by dividing body weight by height [(kg) / (m2)]. Since the adult height will remain constant, the increase in body weight shows an increase in fat mass. BMI is defined as 25 to 29.9 overweight, 30 to 39.9 obese, more than 40 deadly obese. In short, obesity is the balance in favor of calorie intake between calories intake and calories consumed (6). The development of obesity is associated with a significant change in adipose tissue structure. The plasticity of the adipose tissue reflects its extraordinary ability to expand or its size throughout the life of the adult, and the expansion of adipose tissue is closely related to vascular development (7). In recent years, as a result of obesity research, adipose tissue has been reported to be an active endocrine organ that secretes many factors (8). These factors include adipokines, appetite and satiety control, fat distribution, insulin sensitivity, insulin release, energy consumption, inflammation, blood pressure, and factors that regulate endothelial functions (9,10). Functional disorder of adipose tissue is one of the important causes of obesity defects. It is characterized by impaired adipose

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tissue function, atherogenic adipokine structure and proinflammatory secretion. At the same time, impaired adipose tissue function is affected by genetic, behavioral and environmental factors. Degradation processes in adipose tissue lead to mitochondrial dysfunction, depressive adipose tissue, hypoxia, ectopic fat accumulation and adipose hypertrophy (4). Angiogenesis is the formation of new vessels from existing vessels. Physiologically, new vascular formation, wound healing, embryo development, menstrual cycle and pregnancy occur as well as diseases such as new vessel formation, abnormal vascularity, collagen tissue disorders, retinopathies and psoriasis (11). Also known as a vascular factor, adrenomedullin (ADM) is a multifunctional regulatory peptide that is secreted by various cell types. Inflammatory cytokines such as tumor necrosis factor-a (TNF-a) and lipopolysaccharide are potent stimulants of ADM synthesis in adipocytes. In addition, ADM synthesis and ADM concentration have been found to increase in obese fat tissue. The possible physiological role of ADM secreted from adipose tissue may be related to the prevention of metabolic syndrome, type 2 diabetes and hypertension, characterized by obesity through its antioxidant and potent vasodilatory effects. It has been reported that ADM synthesis in adipose tissue plays a role in the pathogenesis of obesity-related diseases in obese individuals (12). ADM is synthesized in human adipose tissue such as epididymal (13) and adherent omental tissue (14). Hypoxia-inducible transcription factors (HIF) activate various pathways that regulate cellular metabolism, angiogenesis, proliferation and migration in a low oxygen or hypoxic environment. Disruption of mitochondrial function can be caused by reactive oxygen species, stress and viral infection and are regulated by HIFs (15). HIF transcription factors in the form of heterodimers containing the A and p subunits are HIF-1, HIF-2 and HIF-3. Although the subunit kontrol is controlled by oxygen-induced proteolytic degradation, the subunit β is preserved structurally (16, 17). HIFs have also been reported to play a role in the pathogenesis of various liver diseases (15). Matrix metalloproteinases (MMPs, Matrixins) are zinc-containing endopeptidases involved in extracellular matrix (ESM) metabolism and are responsible for the degradation of other proteins such as collagen, gelatin, fibronectin and laminin. The MMP family has 28 enzymes with different functions. These enzymes are called collagenases, gelatinases, stromelysins, matrilizins, and shingles-linked MMPs and are partially classified as groups or substrates (18, 19). The activity of these enzymes is regulated by tissue inhibitors. Most of the studies have been reported in obesity model studies with MMPs (19). MMPs represent the most known members of proteinases associated with cancer formation. In addition to extra cellular matrix expansion and cancer cell migration functions, MMPs have been reported to regulate signaling pathways that can function in cell growth, inflammation or angiogenesis, and even non-proteolytic destruction (20).

Material and Methods

Five months old Sprague Dawley male rats produced by Inonu University Experimental Animal Production and Research Center were used in the study. The rats were housed in special cages, 12 hours light/dark, ventilated, room temperature 24 °C. Rats other than the obesity group were fed with standard rat died and water. Obesity is desired to create a group of high-fat dietary diet and water was given. The feed and water needs of the rats were monitored daily until the study was finished. Once a week and the same day of the week, rats were weighed. Obesity group was provided with 20-25% weight increase in rats. Low oxygen pressure (Low PO2) was provided in an environment containing 17-18 % oxygen. Normal oxygen pressure (NPO2) group rats were allowed to breathe with normal air for 21% oxygen. Rats were kept in low oxygen pressure environment for 24 hours. The carbon dioxide formed due to respiration was removed by the soda-lime, which was put in the cage.

Table 1. Rat groups used in the study

Group-I	Standard diet/Normal P_{O2} (SD/N_{O2})
Group-II	Standard diet/low P_{O2} (SD/l_{O2})
Group-III	High-fat diet/Normal P_{O2} (HFD/N_{O2})
Group-IV	High-fat diet/low P_{O2} (HFD/l_{O2})

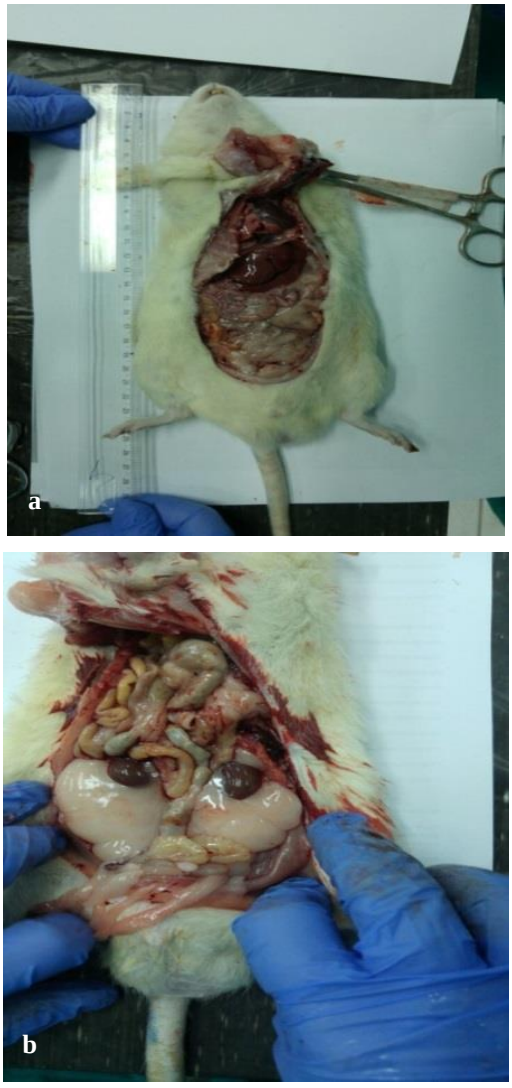

Figure 1. Changes observed during tissue removal from rats. a) Standard diet, b) High-Fat Diet

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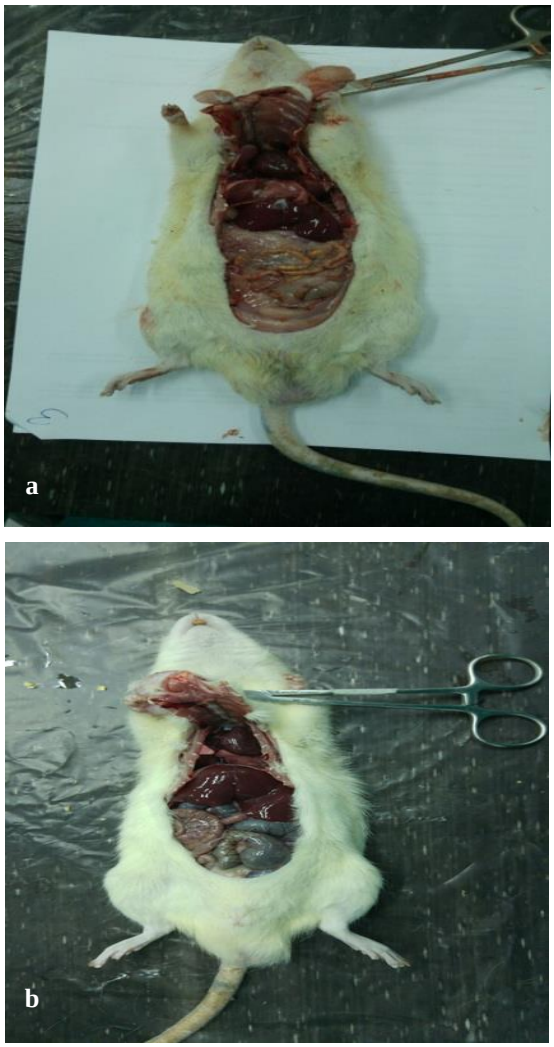

Figure 2. Comparison of normal (a) and hypoxia-exposed rat images (b).

Results

In all working groups; ADM, HIF1-α and MMP-II quantities were measured by ELISA method in liver, lung, WAT, BAT and plasma. Amounts of ADM measured in tissues are shown in Table 2, HIF1-α amounts in Tables 3 and MMP-II in Tables 4.

The greatest increase in ADM levels observed in white adipose tissue, high-fat diet/low oxygen treated group (Table 2). There was no significant increase ($p>0.05$) between SD/lPO2 and HFD/NPO2 groups compared to the ADM levels of liver tissue. Also, there was no significant increase in the amount of ADM in lung tissue and plasma ($p> 0.05$).

According to the results obtained from rat tissues (Table 3), the increase in HIF1-α levels studied in all groups (SD/NPO2, SD/lPO2, HFD/NPO2 and HFD/lPO2) and tissues (liver, lung, WAT, BAT and plasma) ($p <0.05$). When we looked at Table 3, there was a significant increase between SD/NPO2 and HFD/lPO2 groups ($p <0.05$). There was no significant increase between SD/lPO2 and HFD/NPO2 groups ($p> 0.05$).

There was a significant difference between SD/lPO2, HFD/NPO2 groups and SD/NPO2 and HFD/lPO2 groups ($p> 0.05$). There was no significant increase in plasma HIF1-α levels among the groups ($p> 0.05$).

According to the data in Table 4, MMP-II levels studied in all groups (SD/NPO2, SD/lPO2, HFD/NPO2 and HFD/lPO2) and tissues (liver, lung, WAT, BAT and plasma). The significant increase in the amount of MMP-II was found in the WAT and BAT ($p <0.05$).

There was no significant increase in the amount of MMP-II in the liver ($p> 0.05$). There was no significant difference between the SD/lPO2 and HFD/NPO2 groups in the lung tissue ($p <0.05$), whereas the SD/NPO2 group in the lung tissue showed a significant increase in the amount of MMP-II compared to the HFD ($p< 0.05$).

There was a significant increase in the amount of MMP-II between the HFD/lPO2 group and the other three groups (SD/NPO2, SD/lPO2, HFD/NPO2) ($p <0.05$). There was also a significant difference between SD / NPO2 and SD/lPO2 groups ($p <0.05$).

Plasma HFD/lPO2 group showed a significant increase when compared with the other groups ($p <0.05$). There was no significant difference in the increase of MMP-II levels between the other three groups (SD/NPO2, SD/lPO2, HFD/NPO2) ($p> 0.05$).

Table 2. Adrenomedullin (ADM) levels in rat tissues (ng/l). The differences between the different words in the columns are statistically significant. The results are given as mean ± SE.

	Liver	Lung	WAT	BAT	Plasma
SD/NP_{O2}	130.08±3,04^{a}	79.99±2,76^{a}	182.12±13,08^{a}	104.53±5,38^{a}	53.44±1,68^{a}
SD/LP_{O2}	142.41±2,78^{b}	83.21±4,83^{a}	203.56±7,69^{b}	130.36±2,38^{b}	56.34±1,93^{a}
HFD/ NP_{O2}	143.77±6,79^{b}	81.35±4,79^{a}	229.33±12,11^{c}	140.13±4,13^{c}	56.80±2,57^{a}
HFD/LP_{O2}	167.8±6,73^{c}	83.88±8,21^{a}	274.45±10,75^{d}	186.71±0,91^{d}	58.35±6,04^{a}

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Table 3. Hypoxia-inducible factor 1-α (HIF1-α) levels in rat tissues (pg/L). The differences between the different words in the columns are statistically different. The results are given as mean ± SE.

	Liver	Lung	WAT	BAT	Plasma
SD/NP$_{O2}$	66.65±1.99^{a}	32.95±0.70^{a}	43.46±3.49^{a}	50.29±4.84^{a}	30.13±1.51^{a}
SD/LP$_{O2}$	102.35±3.36^{b}	45.20±1.87^{b}	57.15±4.96^{b}	66.08±3.15^{b}	32.71±0.86^{a}
HFD/NP$_{O2}$	95.69±4.38^{c}	52.79±1.33^{c}	74.99±2.99^{c}	59.23±2.44^{b}	30.41±1.33^{a}
HFD/LP$_{O2}$	117.14±3.72^{d}	64.19±3.51^{d}	95.24±3.01^{d}	90.12±4.45^{c}	37.79±2.89^{a}

Table 4. Matrix metalloproteinase-II (MMP-II) level in rat tissues (ng/L). The differences between the different words in the columns are statistically different. The results are given as mean ± SE.

	Liver	Lung	WAT	BAT	Plasma
SD/NP$_{O2}$	23.60±0,45^{a}	20.25±0,36^{a}	22.47±2,21^{a}	25.92±0,97^{a}	18.22±0,32^{a}
SD/LP$_{O2}$	24.31±0,94^{a}	21.13±0,23^{b}	32.05±2,43^{b}	27.01±0,73^{b}	18.48±0,38^{a}
HFD/ NP$_{O2}$	22.94±1,21^{a}	20.47±0,21^{ab}	38.27±1,88^{c}	30.02±0,51^{c}	18.62±0,74^{ab}
HFD/LP$_{O2}$	24.53±0,92^{a}	23.23±0,46^{c}	48.94±2,78^{d}	33.29±0,97^{d}	20.32±0,30^{b}

Discussion

In our study, it was determined that the ADM amount was more increased in obese (HFD/NPO2 and HFD/lPO2) groups compared to the other groups (SD/NPO2 and SD/lPO2) in WAT and BAT. Shibasaki et al. (2010) found that epicardial adipose tissue ADM mRNA levels were elevated in patients with coronary disease in their studies, suggesting that this was a protective effect (21). We also found ADM levels increased in our study. It has been reported that specific binding sites of ADM were mostly in the lung (22). The increase in the amount of ADM in the WAT and HFD / LPO2 group, the onset of angiogenesis in obesity and the increased amount of ADM in the liver also suggest that it may be related to some obesity-related diseases. HIFs activate various pathways that regulate cellular metabolism, angiogenesis, proliferation, and migration in order to be able to respond to a cell in a low-oxygen or hypoxic environment (22). Low oxygen exposure has been reported to be sufficient to induce a hypoxic response that compensates for HIFs (17- 9). Several etiologies of HIFs have been reported to play a role in the pathogenesis of liver disease (16). In our study, an increase in HIF1-α quantities was observed in all tissues except plasma. The increased amount of HIF1-α indicates that HIF has a protective role against obesity in obesity and hypoxia conditions and plays a role in maintaining body homeostasis in hypoxic conditions. At the same time, an increase in the amount of HIF1-α in WAT and HFD, an occurrence of angiogenesis in obesity, and an increase in the amount of HIF1-α in the liver also correlate with some diseases associated with obesity. MMPs are enzymes involved in other proteins such as collagen, gelatin, fibronectin and laminin (23). MMPs represent the most prominent family of proteinases associated with tumor formation. Recent developments have made it clear that MMPs have a prominent role as micro-modulators of tumor. In our study, three angiogenic factors (ADM, HIF1-α and MMP-II) were investigated in five different rat tissues. According to the data we obtained from our study; It was determined that ADM, HIF1-a and MMP-II amounts increased. It was determined that the most significant increase was in WAT.

This indicates that angiogenic factors in obesity, diagnosis and / or treatment pathways will also play an important role. In our study, it was found that MMP-2 activity in WAT and BAT increased significantly compared to rats in the standard diet and normal oxygen group. Based on these evidence, we think that this increase is important in counteracting the adverse conditions arising in the hypoxic state and will positively affect obesity. WAT development can be one of the right approaches to prevent obesity. Therefore, it is unclear which negative or positive angiogenesis regulator can be used in the treatment of obesity.

Conclusion

If the angiogenic vascularization of metabolically active adipose tissue, BAT, increases, it consumes more energy. On the contrary, it has been suggested that inhibition of angiogenesis may be more beneficial in obese individuals with large amounts of metabolically inactive WAT.

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Author's Contributions: MD, MY: concept, design, literature search, data analysis, manuscript preparation, **MY:** manuscript revision, statistical analysis, data acquisition, data analysis

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